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EFFECT OF INSULIN TO DECREASE GLUCOSE TRANSPORT IN DISSOCIATED CELLS FROM THE R3230AC MAMMARY ADENOCARCINOMA OF DIABETIC RATS

JOAN THILLY HARMON[a] and RUSSELL HILF[a, b]

[a]*Department of Biochemistry and* [b]*University of Rochester Cancer Center, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642, N.Y. (U.S.A.)*

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SUMMARY

Dissociated cells of the R3230AC mammary tumor were found to take up glucose by diffusion and by a passive carrier system. Using labeled 3-*O*-methylglucose as the probe, the following properties of the passive carrier were identified: (1) specificity for glucose, (2) competition by galactose and mannose but not by mannitol and fructose, (3) inhibition by phloretin but not by phloridzin, (4) temperature sensitivity, and (5) a K_m for transport of 3–4 mM. The effects of insulin in vitro on carrier-mediated glucose transport were investigated in tumor cells from diabetic rats. At 10^{-9} M insulin, a time-related decrease in v for transport was observed resulting in an increased calculated K_m (2- to 3-fold increase after 60–90 min incubation with insulin); only slight effects on V were obtained. This unusual response in v to insulin was observed when glucose was present in the medium at 2 mM and 5 mM, but not at 20 mM glucose. The effect of insulin to decrease the v was dose-related, with the major effects seen between 10^{-10} M and 10^{-8} M. The apparent decrease in glucose entry in vitro may in part explain the ability of insulin to inhibit growth of this tumor in vivo.

INTRODUCTION

The R3230AC mammary adenocarcinoma, a transplantable tumor of Fischer rats, has been classified as a hormonally autonomous-responsive neoplasm. This classification was based on its biological behavior, as the tumor grows well in the absence of endogenous hormones (oophorectomy, adrenalectomy, orchiectomy or hypophysectomy) but growth of the neoplasm can be inhibited by administration of pharmacologic doses of estrogen, androgens, prolactin or glucocorticoids [1]. An interesting property of this adenocarcinoma was its well-differentiated state as exemplified by its ability to synthesize casein, lactose, α-lactalbumin and shorter chain fatty acids, products found exclusively in the mammary gland during pregnancy and lactation [2]. The secretory response of the carcinoma was induced by treatment with estrogen and was antagonized by concomitant administration of actinomycin D, cycloheximide or the anti-estrogen, MER-25 [1, 2].

Insulin was required by the normal mammary gland in explant culture in vitro. The hormone was shown to induce a wave of DNA synthesis, which was concluded to be a prerequisite for the actions of prolactin and hydrocortisone for the stimulation of casein synthesis [3]. Studies were conducted to determine the role of insulin on the growth and biochemistry of the R3230AC tumor [4]. This neoplasm was found to grow more rapidly in diabetic rats than in intact animals whereas intact animals treated with 2 I.U. insulin per day demonstrated decreased tumor growth compared to untreated (control) tumor-bearing rats. These observations suggested an unusual effect of insulin on the growth of this tumor. To elucidate the effects of insulin on the R3230AC carcinoma, studies were conducted in vitro to characterize the glucose transport systems in the neoplasm and to investigate any effects that insulin would have on these systems. For these studies, dissociated tumor cells were employed; preliminary results have been reported earlier [5].

Prior to examining the effect of insulin on glucose transport, it was necessary to identify the membrane transport components for glucose (facilitated*, active and/or diffusional**) and their characteristics with regard to saturability and specificity. Data presented here demonstrate the existence of both a facilitated carrier and a diffusional glucose transport system, the former showing characteristics comparable to those seen in normal cells [6, 7]. The effect of insulin on glucose transport was examined with respect to the time-course of exposure and to the concentration. Insulin produced a decrease in the initial velocity of glucose transport; this unusual effect, observed in vitro, was both dose-responsive and time-related to the presence of insulin.

MATERIALS AND METHODS

Animals and induction of diabetes

Female Fischer rats (80–90 g), obtained from Charles River Breeding Laboratory (Wilmington, Mass.), were individually housed and offered food and water ad libitum. The R3230AC tumor was implanted subcutaneously in the axillary region on both sides by a sterile trochar technique as described by Hilf et al. [8]. Animals were killed by cervical dislocation at two to three weeks after tumor transplantation.

Diabetes was induced by intravenous injection of streptozotocin one week prior to tumor implantation [4]; diabetes was confirmed by blood glucose levels > 250 mg/100 ml and urinary glucose concentrations exceeding 0.5 mg/100 ml. At necropsy, serum insulin levels were $< 2 \cdot 10^{-10}$ M as determined by radioimmunoassay [9].

Preparation of cells

Tumors were excised from diabetic animals as quickly as possible and placed in chilled 0.9 % saline. The wet weight of the tumors removed from a diabetic animal was approximately 6–10 g (two to three weeks after tumor implantation). Connective

* Passive carrier transport, used here, is also referred to as equalizing selective transport, facilitated transfer, facilitated diffusion, assisted diffusion and carrier-mediated transport.

** The term diffusional component is used here in a conventional sense and in no way presumes that lack of saturation within an accessible concentration range or inhibition by phloretin conclusively demonstrates simple diffusion.

tissue and necrotic areas of the tumor were removed. Three grams of selected tissue were weighed and minced into 1×1 mm pieces on a McIlwain Tissue Slicer (Brinkmann Instruments). The minced tissue was incubated in a 250 ml flask with 10 ml Hank's balanced salt solution ($-Ca^{2+}$ and $-Mg^{2+}$), containing 0.1 % hyaluronidase (Sigma) and 0.05 % collagenase (type II, Worthington), for 1 h at 37 °C in a Benco shaking water bath (≈ 50 cycles/min) [10]. After the first hour, the incubation mixture was poured through a 100 mesh stainless steel strainer. The non-dissociated tissue remaining on the strainer was returned to the flask with a fresh 10 ml aliquot of enzyme solution and incubated for a second hour. The filtrate from the first incubation mixture was discarded because of considerable contamination by red blood cells and cellular debris. At the end of the second hour, the incubation mixture was again strained, and the non-dissociated pieces were rinsed with 10 ml of Medium 199, without glucose (GIBCO). The filtrates from the incubation and wash mixtures were centrifuged for 5 min at ≈ 80×*g*. The cell yield from the second incubation was ≈ 1.6 ml of packed cells from 3 g tumor. The cells from the second incubation were washed at least three times with Medium 199, without glucose. After the final wash, the cells were diluted five-fold with Medium 199, without glucose. Cell viability was monitored by trypan blue exclusion; preparations used in these experiments had greater than 85 % viability. Cell number was estimated by the use of a hemocytometer.

Insulin binding assay

Insulin binding was assayed at 20 °C in a total volume of 1.0 ml Medium 199 with glucose, utilizing 10^6 cells, 1 % bovine serum albumin (Lot 55, Kupits, Forked River, N.J.), and radioactively labeled insulin (^{131}I, 20–50 Ci/g), either alone, or with unlabeled insulin (10^{-6} M). Plastic tubes were employed to minimize adsorption of insulin to the reaction vessel. After the appropriate incubation, 10 ml ice-cold 0.9 % saline were added to the sample, the sample was centrifuged (900×*g*) for 2 min, the supernatant was discarded, the test tube wiped to remove remaining droplets of supernatant, and the cell pellet was counted in a Packard Auto-Gamma Counter, Model 5220 (efficiency for ^{131}I = 30 %).

Glucose transport determination

Glucose transport was measured at 20 °C with 3-*O*-methyl-D-[1-^{3}H]glucose (1 Ci/mmol, Amersham/Searle). Five million cells were suspended in Medium 199 (without glucose) with 1 % bovine serum albumin; 3-*O*-methyl-[1-^{3}H]glucose (3 μCi/vial) plus unlabeled 3-*O*-methylglucose was added to give a final volume of 1 ml. The diffusional component of total transport was determined by the inclusion of 100 μl 4 mM phloretin, which specifically inhibits passive carrier transport [11]. Mannitol was added to maintain uniform osmolarity of ≈ 350 mosM. Transport was halted by addition of 10 ml ice-cold 0.9 % saline followed by centrifugation for 1 min at 3 °C (900×*g*). The cells were rapidly washed once with 10 ml ice-cold 0.9 % saline, centrifuged, and the test tube wiped to remove adhering droplets. The cell pellet was dissolved in two 5 ml aliquots of Aqueous Counting Scintillant (Amersham/Searle) and the sample was counted in a liquid scintillation counter (Nuclear Chicago, Isocap 300) with an efficiency of 37 %.

RESULTS

Characterization of glucose transport systems

The initial velocity (v) of glucose transport in the R3230AC tumor cells was determined, using labeled 3-*O*-methylglucose. Samples were obtained at several time points over a 15 min period; results are illustrated in Fig. 1. It was observed that transport of 3-*O*-methylglucose in vitro demonstrated a linear relationship with time for the first 1.5–2 min, after which time divergence from linearity was seen. Phloretin, an inhibitor of passive carrier transport [11], inhibited 3-*O*-methylglucose entry and phloridzin, which specifically inhibits active glucose transport [13, 14], did not inhibit 3-*O*-methylglucose entry (Table I). Phloretin was employed to provide an estimate of

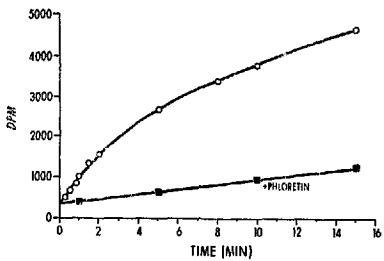

Fig. 1. Time course of 3-*O*-methylglucose (5 mM) transport in the absence and presence of phloretin (200 μM) at 20 °C. ○, total 3-*O*-methylglucose transport; ■, 3-*O*-methylglucose transport in the presence of phloretin (diffusional transport).

TABLE I

3-*O*-METHYLGLUCOSE TRANSPORT IN THE PRESENCE OF COMPETITORS OR INHIBITORS

Transport of 3-*O*-methylglucose (1 mM) was measured for 60 s at 20 °C. Competitors (10 mM) or inhibitors (400 μM) were added at zero time in presence of 3-*O*-methylglucose.

	% 3-*O*-methylglucose transport
Competitor	
Mannitol	106
Fructose	99
Galactose	56
Mannose	40
Glucose	22
Inhibitor	
Phloridzin	96
Phloretin	8

the diffusional component of total glucose transport. As expected, diffusional entry of glucose was linear over the entire 15 min time course studied and represented $\approx 10\,\%$ of total transport at 1 mM 3-*O*-methylglucose. The difference between total 3-*O*-methylglucose transport and transport in the presence of phloretin was calculated and represented a measure of passive carrier transport [12]. All subsequent experiments on glucose transport were conducted at 15 s intervals for a total of 60 s, a time period during which transport was linear*.

To characterize the passive carrier system, competition and inhibition studies were carried out using 1 mM 3-*O*-methylglucose and either 10 mM competitor or 400 μM inhibitor (Table I). Addition of mannitol or fructose demonstrated no competition for glucose transport, whereas addition of either galactose, mannose or glucose demonstrated competition for 3-*O*-methylglucose transport in these tumor cells. The slight decrease in 3-*O*-methylglucose entry in the presence of 400 μM phloridzin could be attributed to the $\approx 5\,\%$ contamination of phloridzin with phloretin**.

Data presented in Table II indicate that 3-*O*-methylglucose transport into tumor cells was found to be temperature-dependent.

From initial velocity measurements, the kinetic constants, K_m and V, were calculated***. Total 3-*O*-methylglucose transport was composed of two components, passive carrier and diffusional transport, as defined by the following equation:

$$v^T = v^C + v^D = \{V[S]/(K_m + [S])\} + K_D[S]$$

where v^T = initial velocity of total transport; v^C = initial velocity of passive carrier transport; v^D = initial velocity of diffusional transport; V = maximum velocity of passive carrier; [S] = 3-*O*-methylglucose concentration; K_m = Michaelis-Menten constant for passive carrier; and K_D = diffusional coefficient. In the above equation, it was assumed that passive carrier transport followed Michaelis-Menten kinetics, whereas diffusional transport was a linear function of substrate concentration [12].

Since it was possible to estimate the diffusional transport by using the inhibitor phloretin, the values for v^D were subtracted from v^T to yield v^C. The data were plotted in two ways; v^T vs. [S] and v^C vs. $v^C/[S]$ (Fig. 2). Only the v^C was plotted according to Eadie-Hofstee, from which were obtained both the K_m (-slope of line) and V (intercept on the ordinate). The best fitting straight line on the Eadie-Hofstee plot was determined by linear regression analysis.

Degradation of insulin in vitro

Insulin is known to be degraded by cells and purified membranes in vitro [15, 16]. To investigate degradation of insulin by the dissociated tumor cells studied here, the effects of bovine serum albumin added to the incubation medium were examined. When $5 \cdot 10^6$ cells were employed, addition of bovine serum albumin to a final concentration of 1 % provided conditions in which the degradation of insulin

* Correction for trapping or adsorption of labelled 3-*O*-methylglucose to the cell pellet was not necessary because the initial velocity was determined from the slope of the line and is independent of the intercept on the ordinate (see Fig. 1).

** Chromatographic analysis revealed the presence of phloretin ($\sim 5\%$) in the samples of phloridzin used (Kimmich, G., personal communication).

*** Some variation in V, but not K_m, was observed with different lots of collagenase and/or hyaluronidase.

TABLE II

EFFECT OF TEMPERATURE ON 3-*O*-METHYLGLUCOSE TRANSPORT

Concentration of 3-*O*-methylglucose was 1 mM.

Temperature (°C)	v (μmol/min/10^6 cells) × 10^{-4}		
	Carrier +Diffusion	Diffusion	Carrier
37	1.8	0.4	1.4
30	1.5	0.3	1.2
25	1.1	0.2	0.9
20	0.4	0.1	0.3

(10^{-9} M) was linear with time and was estimated (by precipitation with trichloroacetic acid) to be 10 % at the end of a 120 min incubation period at 20 °C. Other lots of bovine serum albumin gave variable results; in some instances, 2 % bovine serum albumin was required to achieve the same degree of protection of insulin. To ascertain the biological activity of the remaining insulin, experiments were conducted in which cells were incubated with 10^{-9} M insulin for 30 min under conditions used for glucose transport studies, the cells were removed, and the medium was added to fresh cells. Glucose transport was measured after an additional 30 min incubation period. The effects of this "conditioned medium" on glucose transport, i.e., decrease in v^C, were identical to those seen with freshly added insulin to cells and incubated for 30 min. The data indicate that the extent of degradation of insulin during a 60 min incubation period was not sufficient to alter the hormonal effect on glucose transport.

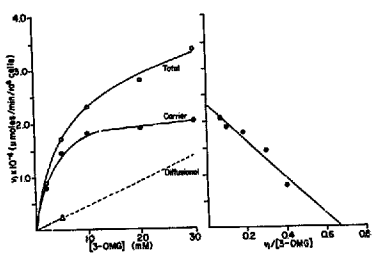

Fig. 2. Left: Initial velocity of 3-*O*-methylglucose (3-OMG) transport at 20 °C. The diffusional component was estimated by inclusion of 400 μM phloretin to 5 mM 3-*O*-methylglucose. The difference between total transport and diffusional transport gave the passive carrier transport. Mannitol was added to maintain a constant osmolarity of 350. v, initial velocity; ○, v^T; ●, v^C; and △, v^D. Right: Data for passive-carrier transport (v^C) plotted according to Eadie-Hofstee. Line was determined by linear regression analysis (correlation coefficient = 96 %). K_m = 3.5 mM; $V = 2.3 \cdot 10^{-4}$ μmol/min/10^6 cells.

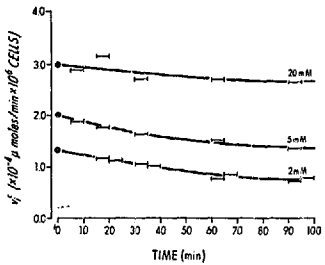

Fig. 3. The effect of incubation with insulin (10^{-9} M) on 3-*O*-methylglucose transport in dissociated R3230AC tumor cells. Three concentrations of 3-*O*-methylglucose are shown which simulate hypoglycemia (2 mM), normoglycemia (5 mM) and hyperglycemia (20 mM) in vivo. The horizontal bars represent the 5 min necessary to perform the transport measurements. The times shown correspond to the period of incubation with insulin; v^C, initial velocity of the passive carrier.

Effect of insulin on glucose transport

To investigate the effect of insulin on glucose transport in tumor cells, experiments were conducted initially to determine the effects of different times of incubation of cells with insulin in vitro on the transport of 3-*O*-methylglucose. The data presented in Fig. 3 show the effect of incubating tumor cells in the presence of 10^{-9} M insulin for various time periods prior to measuring transport using three concentrations of 3-*O*-methylglucose. Since the experimental design for measurement of transport required 5 min for completion, the data for v^C are presented for the overall 5 min time interval. The v^D remained constant for 90 min and was subtracted from the v^T to yield the data shown. The initial time point (0 min) corresponded to the initial velocity of the passive carrier transport in the absence of insulin. In the absence of insulin, the initial velocity remained constant over the 90 min interval. Thus, in the R3230AC tumor, insulin had the effect to decrease the passive carrier transport system (v^C); the extent of response to insulin was related to the time of exposure of the tumor cells to the hormone.

These data were also analyzed according to Eadie-Hofstee and the kinetic constants were obtained at 0, 15, 30, 60 and 90 min of exposure to insulin (Table III). An increase in K_m with time was observed. There appeared to be a slight increase in V during the first 30 min of exposure to insulin; however, this was not seen at 60 and 90 min.

Utilizing 2 mM 3-*O*-methylglucose, the concentration of insulin (10^{-11}–10^{-7} M) was varied to determine the effect of dose on 3-*O*-methylglucose transport (Fig. 4). The transport studies were done after the tumor cells were incubated for 0, 15, 30, 60 and 90 min in the presence of different concentrations of insulin. For these experiments, the data were plotted as the change in v^C for a given time and insulin concentration, according to the following relationship: $-\Delta v^C = v^C$ (plus insulin) $- v^C$ (minus insulin). Since the observed effect of insulin was to decrease the initial velocity, the Δv^C had a negative value. The data demonstrated that the maxi-

TABLE III

EFFECT OF INSULIN (10^{-9} M) ON KINETIC PARAMETERS OF 3-*O*-METHYLGLUCOSE TRANSPORT IN R3230AC TUMOR CELLS

Incubation with Insulin (min)	K_m (mM)	V (μmol/min/10^6 cells) $\times 10^{-4}$	Correlation coefficient*
0**	3.2	3.4	0.95
5	4.2	3.5	1.00
15	4.6	3.7	0.97
30	5.2	3.6	0.92
60	6.8	3.6	0.96
90	7.2	3.4	0.94

* Correlation coefficient for linear regression analysis of Eadie-Hofstee plot.
** Kinetic parameters as determined in the absence of insulin in vitro.

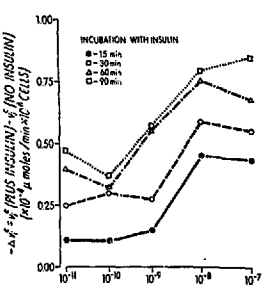

Fig. 4. The effect of the concentration of insulin on the initial velocity of the passive carrier system as measured with 2 mM 3-*O*-methylglucose. Data are expressed as the difference in v^c observed in the presence or absence of insulin; cells were incubated for 15, 30, 60, and 90 min with various amounts of insulin. In the absence of insulin, v^c was $1.07–1.52 \cdot 10^{-4}$ μmol/min/10^6 cells.

mum effect was achieved at 10^{-8} M insulin. The dose-related effect of insulin was observed between 10^{-10} M and 10^{-8} M.

Previously, we had measured insulin binding to those tumor cells [5]. It was of interest, therefore, to compare the time course of insulin binding with the time course of 3-*O*-methylglucose transport in the presence of 10^{-9} M insulin (Fig. 5). For this comparison, the data obtained at 90 min were arbitrarily set at 1.0 (100 %) since the time course of specific insulin binding demonstrated a plateau at 60–90 min. In the case of 3-*O*-methylglucose transport, the $-\Delta v^c$ at 90 min was also set at 1.0 (100 %) and the $-\Delta v^c$ at each of the other time points was calculated in relationship to the $-\Delta v^c$ at 90 min (e.g., $\{-\Delta v^c$ (15 min) divided by $-\Delta v^c$ (90 min)$\} \cdot 100 =$ % change in initial velocity at 15 min with respect to change in initial velocity at 90 min).

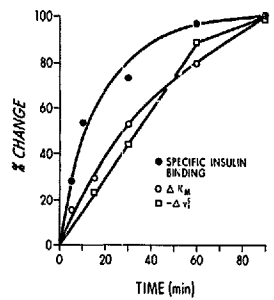

Fig. 5. Temporal relationship of insulin binding (10^{-9} M) and insulin effects on glucose transport (3-*O*-methylglucose) in R3230AC carcinoma cells in vitro. Values obtained at 90 min of incubation were set at 100 %. Data for K_m were calculated from experiments utilizing 2, 5, 10, 20 and 30 mM 3-*O*-methylglucose (see Fig. 2); change in v^c was calculated from studies utilizing 2 mM 3-*O*-methylglucose (see Fig. 3).

Binding of insulin occurred rapidly, reaching ≈ 50 % of the maximum by 10 min; maximum binding occurred within 60 min. The effect of insulin to decrease the v^c of 3-*O*-methylglucose transport was not as rapid as insulin binding; ≈ 50 % of the maximum effect was observed at 30 min. The time-related effect of insulin binding displayed hyperbolic characteristics, whereas the effect on glucose transport (decrease in v^c) appeared to be linear during the first 60 min. A hyperbolic relationship (similar to insulin binding) was seen for the effect of insulin on the K_m of 3-*O*-methylglucose transport. The results suggest that a temporal relationship exists between insulin binding and glucose transport, with the hormonal interaction preceding the response induced on glucose transport.

DISCUSSION

The R3230AC tumor has previously been shown to rapidly metabolize glucose through the hexose-monophosphate pathway and the Embden-Meyerhof pathway [4]. It was of interest to investigate transport of glucose, as compared to total uptake of glucose; for this purpose, 3-*O*-methylglucose was utilized. 3-*O*-Methylglucose, which cannot be phosphorylated on entering the cell, is not permanently retained within the cell. Thus, it is essential to determine 3-*O*-methylglucose transport as early as possible before a sufficient amount has accumulated within the cell, leading to back-diffusion. This was accomplished by performing experiments during the time that transport of 3-*O*-methylglucose was linear (Fig. 1); this enabled us to measure the rate of 3-*O*-methylglucose transport.

It should be noted that throughout these experiments, we also observed an effect of bovine serum albumin. Although the effects of insulin and diabetes were independent of both the source and lot of bovine serum albumin used, the absolute

values calculated for the kinetic constants varied to some extent.

One purpose of this investigation was to measure and characterize glucose transport systems in R3230AC tumor cells and then to determine if these systems responded to insulin. The glucose transport system showed specificity for D-glucose and certain other hexoses with structural similarities to glucose. The data indicated that 3-*O*-methylglucose entered the cell both by diffusion and by passive carrier. The diffusional transport component was defined (with the reservations mentioned earlier) on the basis that it showed linearity with time and substrate concentration over the range of 1 to 30 mM 3-*O*-methylglucose.

In contrast, the passive carrier transport component was defined on the basis that it was a saturable process and that it was inhibited by phloretin. Since phloridzin (up to 400 μM) did not inhibit transport, it was concluded that an active transport component was probably not present. Total transport was a temperature dependent process; passive carrier transport demonstrated the major temperature dependency.

The values of the kinetic parameters, K_m and V, of the passive carrier system cannot be determined precisely because determination of the diffusional system cannot be done with absolute accuracy. To best evaluate the kinetic parameters, therefore, phloretin was used to block the passive carrier; this approach yielded data to estimate glucose entry by diffusion. Similar techniques, i.e., use of inhibitors to block the passive carrier, have been utilized by others in the determination of glucose transport systems in brown fat cells [6] and Novikoff rat hepatoma cells [19]; the values for K_m, reported by these investigators, were similar to those presented here.

The effects of insulin on glucose transport have been investigated in both white and brown fat cells [17, 6, 20], muscle [18, 7], erythrocytes [21] and other tissues (see review by Elbrink and Bihler, [22]). In most instances, insulin either increased or had no effect on glucose uptake. The data presented here, in contrast, demonstrated that insulin decreased the v^c by approximately 40 % at 2 mM 3-*O*-methylglucose after exposure of tumor cells to 10^{-9} M insulin for 60–90 min. This decrease was based on comparing the v^c of dissociated tumor cells not incubated with insulin, which represented the control. While it is desirable to utilize a normal cell and then contrast normal with abnormal, a question arises as to what would be the best choice for normal. Adipose tissue, which comprises about 90 % of the mammary gland from the virgin rat, does not reflect the cell composition of the tumor. A better choice would be the gland during lactation, which contains a predominance of epithelial cells; as such, it is more like the composition of the tumor which contains little or no adipose tissue. O'Keefe and Cuatrecasas [23] reported that epithelial cells from pregnant animals were not responsive to insulin in vitro, although they only measured glucose utilization and not transport per se; their studies were performed on tissues from intact animals. The unusual effects of insulin seen here to inhibit glucose transport may in part be due to our use of tumors from diabetic rats since the response was enhanced compared to that seen in tumors from non-diabetic animals [5]. However, the insulin effect was shown to be related both to the time of exposure to insulin and to the concentration of insulin. Such results are usually anticipated for a hormonal response.

Studies of insulin binding to R3230AC tumor cells will be presented in detail elsewhere*. However, for the purpose of comparing the time course of insulin binding

* Harrison, J. T. and Hilf, R., manuscript in preparation.

with the time course of 3-*O*-methylglucose transport in the presence of 10^{-9} M insulin, some of the specific binding data were presented in Fig. 5. A relationship between the binding of insulin and its effect on glucose transport was observed. However, the two plots are not superimposable especially during the first 30 min. One possible explanation for the delay in response is that another event, such as ion flux, e.g., Ca^{2+}, or adenyl cyclase activation or inactivation may be necessary for the onset of the biological response, thereby causing the glucose transport events to be delayed. Elucidation of the mechanism must await further experimentation.

The ability of insulin to influence the biological behavior of the R3230AC tumor in vivo may be explained in part by the effects observed on glucose transport in vitro. First, specific receptors for insulin in the R3230AC carcinoma were demonstrated to have properties similar to those in normal cells [24]. Second, data shown here indicate the presence of a passive glucose transport system in the carcinoma possessing kinetic properties similar to those reported for other cells. Third, insulin induced a paradoxical response in the passive transport system with the hormone causing a reduction in v_e leading to an increase in the calculated K_m. Since administration of insulin decreased tumor growth in intact animals, the combined effects of inhibition of glucose transport, decreased hexose monophosphate pathway activity and lack of stimulation of hexokinase activity [4] could lead to decreased substrate availability and utilization. The more rapid growth of the R3230AC tumor in diabetic rats may be a result of the increase in v^e of glucose transport plus the increased diffusional influx of glucose in the hyperglycemic state; these effects would more than offset the modest decrease ($\approx 20\,\%$) in hexokinase activity and the slight decrease in hexose monophosphate pathway utilization of glucose [4]. Obviously, several assumptions were made by comparing tumor growth in vivo, enzyme activity measured under optimum conditions, substrate utilization by tumor slices in vitro and glucose transport by dissociated cells in vitro. Nevertheless, the data do not reveal any striking disparities and provide us with a working hypothesis to examine in future experiments.

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